American Journal of Hematology 53:35–36 (1996)

Clinical, Genetic, and Biochemical Features of G-6PD$^{West\ Virginia}$

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A new mutation of the G-6PD gene at position 910 is described. Biochemical analysis suggests that a conformational change results in the enzymatic deficiency associated with G-6PD$^{West\ Virginia}$. © 1996 Wiley-Liss, Inc.

Key words: hemolytic anemic, G-6PD, enzymopathy

INTRODUCTION

Functional deficiency of glucose-6-phosphate dehydrogenase (G-6PD), which serves as the first enzyme in the pentose phosphate pathway, results in decreased nicotinamide adenine dinucleotide phosphate (NADPH) levels and leads to oxidant-induced red blood cell hemolysis. With more than 400 variants and 60 mutations known, G-6PD deficiency is the most common enzymopathy [1]. We report here the clinical, genetic, and enzymatic features of G-6PD$^{West\ Virginia}$, a class 1 variant.

CASE HISTORY

A 5-day-old white baby boy was referred to the Children's Hospital for evaluation of severe jaundice and hemolysis. He was a full-term product of a 21-year-old G_1P_0 woman. Delivery was complicated by cord-wrap around the neck, and fetal decelerations. Apgars were 8 and 9 at one and five minutes. Shortly after birth, the baby was noted to be yellow. At age 12 hr, total bilirubin was 14 mg/dl, with a direct fraction of 3.9 mg/ml. Hemoglobin was 9.7 g/dl. Mean corpuscular volume was 117 femtoliters. Uncorrected reticulocyte count was 16.8%. Lactate dehydrogenase level was 350 IU/l. The baby was treated with phototherapy and hydration. Bilirubin levels peaked on day 6 of life, with a total level of 16.7 mg/dl and a conjugated level of 12.5 mg/dl.

The G-6PD activity of the infant's erythrocytes was 0.5 IU/g Hb (normal, 5.0–9.4). The baby was discharged on folic acid. The child is presently 2 years old and has been in good health. He has had two hospitalizations for hemolytic crises, which he has tolerated well without transfusion. On his most recent evaluation, his hemoglobin was 10.5 g/dl, and the uncorrected reticulocyte count was 13.2%.

Two maternal uncles have documented G-6PD deficiency, but their form had not been characterized genetically or biochemically. Both uncles are well, and report infrequent episodes of hemolysis. One underwent cholecystectomy in the fourth decade of life.

GENETIC AND BIOCHEMICAL ANALYSIS

DNA was extracted from peripheral blood of the proband and subjected to single-strand conformation polymorphism analysis and sequencing. A G→T mutation occurred at position 910. This results in a Val→Phe substitution at amino acid 303. Also, a silent mutation, C→T, occurred at nucleotide 1,191. This polymorphism has not been previously found [2].

G-6PD was purified from peripheral blood of the proband's uncle. Using methods recommended by the World Health Organization Scientific Group [3], biochemical analysis of G-6PD$^{West\ Virginia}$ is summarized in Table I. Electrophoretic analysis showed that mobility was normal in Tris, but slower in EBT (EDTA-Borate-Tris) and faster in phosphate. Residual enzymatic activity was 1% of normal. The apparent Km for glucose-6-phosphate was

Received for publication March 12, 1996; accepted April 10, 1996.

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TABLE I. Biochemical Characteristics of G-GPD$^{West\ Virginia}$

G-6-PD	Red cell activity (%)	Electrophoresis (% of normal)			Km (μM)		Utilization (% of normal)		pH optima	Heat stability
		Phosphate	Tris	EBT	G-6-P	NADP	Deoxy G-6-P	Deamino NADP		
Normal	100	100	100	100	40–60	2-4	100	100	Truncate	Normal
West Virginia	1	110	100	98	25.9	6.6	7.8	67	Bimodal	Very unstable

25.9 μM (normal, 40–60 μM), and Km for NADP was 6.6 μM (normal, 2–4 μM). The utilization of deamino NADP was 67% of normal.

DISCUSSION

The molecular basis of this deficiency is a new mutation at nucleotide position 910, which results in 303Val→Phe. This the first case of amino acid 303 being affected. The affected amino acid is not near amino acid 386, where mutations markedly affect the binding of NADP [4]. It is also not near the glucose-6-phosphate-binding lysine at amino acid 205 [5]. Upon biochemical analysis of purified protein, this G-6PD variant is not closely related in its properties to any other variant previously characterized. The deduced mutations does not result in a charge change. Indeed, it did not produce a consistent change in the electrophoretic mobility of the enzyme, which was faster in phosphate and slower in EBT. It is not clear why the substitution of one hydrophobic amino acid for another should result in such a dramatic instability of the enzyme, but it may be that the bulk of the phenylalanine group destabilized the folded structure to produce marked instability, and, consequently, the enzymatic deficiency. It is therefore possible that there is a conformational change which results in the enzymatic deficiency associated with the G-6PD$^{West\ Virginia}$ variant.

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